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Desulfonation of Linear Alkylbenzenesulfonate Surfactants and Related Compounds by Bacteria

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***Pseudomonas putida* S-313 (= DSM 6884) grew in sulfate-free medium when the sole sulfur source supplied was one of several arylsulfonates involved in the synthesis, application, or biodegradation of linear alkylbenzenesulfonate (LAS) surfactants. 2-(4-Sulfophenyl)butyric acid, 4-*n*-butyl-1-methyl-6-sulfotetralin, and 4-toluenesulfonic acid were each completely utilized during growth, as were the model LAS 1-(4-sulfophenyl) octane and the arylsulfonate dyestuff Orange II. The product in each case was the corresponding phenol, which was identified by gas chromatography-mass spectrometry or ^{1}H nuclear magnetic resonance. Stoichiometric conversion of 4-toluenesulfonic acid to 4-cresol was observed. The molar growth yields observed were 2.4 to 2.8 kg of protein per mol of S, which were comparable to the yield for sulfate. Commercial LAS disappeared from growth medium inoculated with strain S-313, but negligible growth occurred; digestion of cells in alkali led to recovery of the LAS mixture, which seemingly sorbed to the cells. However, mixed culture L6 was readily obtained from batch enrichment cultures containing commercial LAS as a sole sulfur source and an inoculum from domestic sewage. Culture L6 desulfonated components of the LAS surfactant to the corresponding phenols, which were identified by gas chromatography-mass spectrometry. Compounds with shorter alkyl chains were desulfonated preferentially, as were the centrally substituted isomers. In the presence of 200 μM sulfate, culture L6 grew well and LAS disappeared, although this was due purely to sorption, as shown by digestion of the cells in alkali. Thus, under sulfate-limited conditions, LAS can be desulfonated directly.**

With one known exception, sulfonated aromatic compounds are xenobiotic (10). These compounds are produced in large amounts as linear alkylbenzenesulfonate (LAS) surfactants (global production, 1.8×10^6 metric tons/year in 1987 [3]) and as dyestuffs ($>3 \times 10^5$ metric tons/year [1]) and in smaller amounts as additives for detergents, oils, and inks. The LAS surfactants are generally considered to be biodegradable (31, 36, 40), but other sulfonoaromatic compounds are well known for their recalcitrant nature (19, 25, 28, 46).

Commercial LAS is a mixture of many different isomers and homologs (Fig. 1A and Tables 1 and 2). It is present in sewage at levels of 1 to 20 mg/liter (13, 32), and most isomers are susceptible to ready biological attack. However, the extent of mineralization is isomer dependent (40), and 11 to 19% of the carbon in some samples has been recovered as nondegraded material (20). Two major families of impurities found in commercial LAS preparations are the dialkyltetralinsulfonates (DATS) (Table 1), which are by-products of the synthetic process (6), and the branched-chain alkylbenzenesulfonates, which may constitute a significant proportion of the surfactant (up to 5% in some preparations [29, 30]). Toluenesulfonate (Table 1) is an additive in some detergent formulations (37) and as such is released into the environment in large amounts. 4,4′-bis(2-Sulfostyryl)biphenyl is a typical representative of the stilbene class of fluorescent optical brighteners and is also used in laundry formulations; like the arylsulfonate aromatic dyestuffs, such as Orange II, this compound is generally persistent in the environment (2, 7, 34).

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For a long time it was believed that desulfonation always occurs early in the aromatic sulfonate degradative pathway (5). However, it has now been established that desulfonation also occurs at later stages (11, 15, 22–24). For LAS surfactants it is generally believed that sulfur is eliminated very late in the normal biodegradative pathway (16, 31, 36, 38) following initial oxidation of the alkyl chain to give carboxylated intermediates, such as sulfophenylbutyrate (Table 1). Sulfonated dyestuffs, if they are broken down at all, are also desulfonated only after initial steps (33, 46).

Zürrer et al. (47) were the first authors to describe a bacterial system that desulfonates a wide range of aromatic sulfonates (cf. 39). Whereas other researchers studied organosulfonates as carbon and energy sources for growth and found narrow substrate ranges, Zürrer et al. (47) examined the sulfonates as sulfur sources for growth. The organism studied in most detail, *Pseudomonas putida* S-313, carries out monooxygenolytic cleavage of the carbon-sulfur bond, yielding the corresponding phenol (47).

This initial desulfonation of the arylsulfonate moiety provides a potential advantage for biodegradation, namely, that the xenobiotic nature of the compound is rapidly reduced, allowing further metabolism of the aromatic ring to be carried out by less specialized microorganisms. In this study we examined the desulfonation of compounds that are either structurally related to LAS or involved in LAS application and thereby released into the environment. In this paper we show that *P. putida* S-313 desulfonates a model LAS, a typical intermediate in the degradation of commercial LAS, a compound added to commercial detergents, an impurity found in commercial LAS, and a sulfonated dyestuff. We also found that another culture, mixed culture L6, desulfonates components of a commercial LAS mixture.

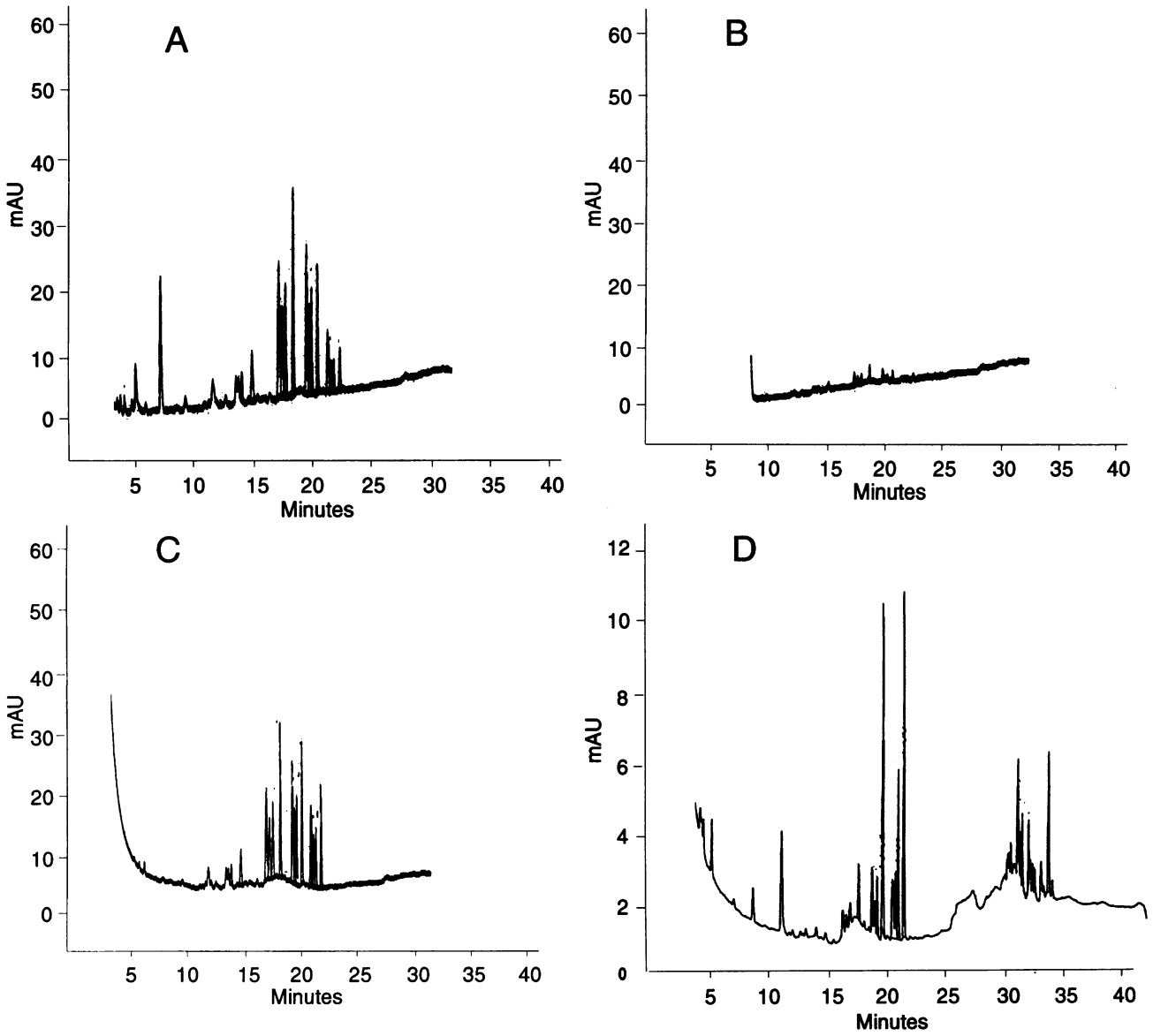


FIG. 1. LAS and LAS desulfonation products in growth medium after growth of mixed culture L6. (A) Commercial LAS, a mixture of about 20 different compounds. (B) Residual LAS in the growth medium after growth of mixed culture L6 in the presence of LAS and sulfate. (C) Same as panel B, after alkali treatment to release cell-bound LAS. (D) Residual LAS and the products of LAS desulfonation (retention times, 30 to 35 min) after growth of mixed culture L6 with LAS as the sole source of sulfur. The products were separated by HPLC as described in Materials and Methods.

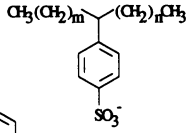
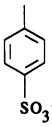
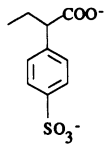
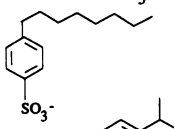
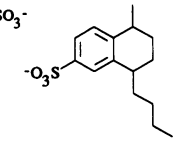
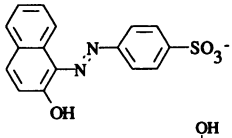
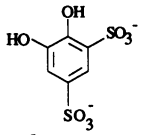
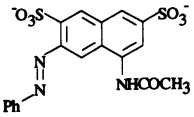
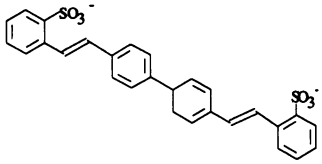
MATERIALS AND METHODS

Organisms and growth media. *P. putida* S-313 (= DSM 6884) (4, 47) was routinely grown in sulfate-free, succinate minimal medium, as previously described (18). Arylsulfonates (50 to 100 μM) were added as sole sources of sulfur, as described below. Growth was monitored by measuring optical density at 650 nm, and cultures were harvested when they reached the stationary phase.

Cultures were enriched for LAS-utilizing organisms under sulfur limitation conditions as previously described (9). Domestic sewage from a municipal activated sludge plant (Teufen, Switzerland) was washed three times with 10 mM potassium phosphate buffer (pH 7.3) and then inoculated (10%, vol/vol) into a sulfate-free minimal medium (9) containing 5 mM glucose, 5 mM succinate, and 10 mM glycerol as carbon sources and commercial LAS as the sole source of sulfur (13 mg/liter; ca. 50 μM). After three subcultures into homologous medium (each 1%, vol/vol) a stable, LAS-utilizing mixed culture was obtained. This mixed culture, culture L6, was subsequently grown routinely in a modified growth medium which contained 50 mM potassium phosphate and 10 mM glucose as the sole carbon source.

Materials and apparatus. 2-(4-Sulfophenyl)butyric acid was synthesized from 2-phenylbutyric acid and was purified by recrystallization from methanol-ether (1:1) (41). 4-*n*-Butyl-1-methyl-6-sulfotetralin (a DATS) was kindly provided by Vista (Austin, Tex.), and 1-(4-sulfophenyl)octane was purchased from Aldrich (Steinheim, Germany). Commercial LAS was

TABLE 1. Arylsulfonates used in this work and utilization of these compounds by *P. putida* S-313 as sulfur sources[a]

Compound	Structure	Environmental relevance	Molar growth yield (kg of protein/mol of S)[b]
LAS[c]		Detergent	No growth[d]
Toluenesulfonate		Detergent additive	2.8
Sulfophenylbutyrate		Intermediate in LAS biodegradation	2.4
Sulfophenyloctane		Model LAS detergent	2.7
DATS[e]		By-product in LAS synthesis	1.4
Orange II		Dyestuff	2.5
Tiron		Chelating agent	2.9
Acid Red I		Dyestuff	No growth
4,4′-bis(2-Sulfo-styryl)biphenyl		Optical brightener	No growth[d]

[a] *P. putida* S-313 was grown in minimal salts medium containing different arylsulfonates at a concentration of 50 μM as the sole sources of sulfur. Growth was monitored by measuring protein formation.

[b] The molar growth yield obtained with inorganic sulfate was 2.8 kg/mol of S. Growth was negligible in the absence of an added sulfur source.

[c] The commercial LAS used was a mixture of C_{10} to C_{13} isomers and homologs (m = 0 to 3; n = 5 to 10) (Table 2).

[d] Substrate disappeared from the culture medium, although no growth was observed.

[e] The DATS found in commercial LAS samples is a mixture of isomers and homologs, corresponding to the LAS composition. In this study the single isomer 4-butyl-1-methyl-6-sulfotetralin was used.

supplied by Tokyo Chemical Industry (Tokyo, Japan), and the optical brightener 4,4′-bis(2-sulfostyryl)biphenyl was obtained from Ciba-Geigy (Basel, Switzerland). All other sulfonates were supplied by Fluka (Buchs, Switzerland). Before they were used, the arylsulfonates containing inorganic sulfate were purified by reversed-phase high-performance liquid chromatography (HPLC), using a Nucleosil-C_{18} column (250 by 4.6 mm; particle size, 7 μm); the mobile phase used was 100 mM potassium phosphate buffer (pH 6.7) containing appropriate amounts of methanol (0 to 80%, vol/vol), and compounds were detected spectrophotometrically at 200 and 224 nm.

Analytical methods. To identify metabolites, stationary-phase cultures (300 ml) were acidified with HCl, saturated with sodium chloride, and extracted three to five times with an equal volume of diethyl ether. The combined extracts were dried over anhydrous sodium sulfate, evaporated, and redissolved in diethyl ether for analysis by gas chromatography-mass spectrometry. For gas chromatography we used a 30-m type DB5 column (J & W Scientific, Folson, Calif.) with an inside diameter of 0.32 mm; the thickness of the film [5% (diphenyl)dimethylpolysiloxane] was 25 μm, the helium flow rate was 1 ml/min, the injection port temperature was 265°C, and the column temperature was increased from 70 to 240°C at a rate of 8°C/min. For mass spectrometry we used a model ITD 800 ion trap detector (Finnigan Mat, San Jose, Calif.). Compounds which were sensitive to the gas chromatography con-

TABLE 2. Analyses of the LAS preparation, its sorption to culture L6, its desulfonation by culture L6, and the product formed[a]

Component(s) of LAS	Amt (%)[b]	Level of sorption (%)[c]	% Utilized[d]	Amt of product formed (relative %)[e]
6- and 5-(4-Sulfophenyl)-$C_{11}H_{23}$	11.4	89	100	100
4-(4-Sulfophenyl)-$C_{11}H_{23}$	7.6	86	100	103
3-(4-Sulfophenyl)-$C_{11}H_{23}$	9.7	88	100	76
2-(4-Sulfophenyl)-$C_{11}H_{23}$	17.6	91	93	43
6- and 5-(4-Sulfophenyl)-$C_{12}H_{25}$	12.5	93	92	51
4-(4-Sulfophenyl)-$C_{12}H_{25}$	7.6	96	94	55
3-(4-Sulfophenyl)-$C_{12}H_{25}$	9.0	96	90	46
2-(4-Sulfophenyl)-$C_{12}H_{25}$	10.7	94	63	26
7-, 6-, and 5-(4-Sulfophenyl)-$C_{13}H_{27}$	5.1	100	87	106
4-(4-Sulfophenyl)-$C_{13}H_{27}$	2.6	100	86	47
3-(4-Sulfophenyl)-$C_{13}H_{27}$	2.6	100	41	0
2-(4-Sulfophenyl)-$C_{13}H_{27}$	3.5	96	0	0

[a] Mixed culture L6 was grown by using commercial LAS as the sole source of sulfur.
[b] Proportion of each component relative to the total amount of LAS.
[c] Sorption to bacteria was measured by determining the percentage of each isomer that disappeared in the presence of 200 μM sulfate, which prevented desulfonation (see text).
[d] The level of LAS utilization was determined after alkaline lysis of the cells to release the bound LAS.
[e] The yield of product was normalized for the amount of each isomer present initially in the LAS mixture. We assumed that the alkyl phenol products had comparable extinction coefficients at 220 nm.

ditions were redissolved in 100 mM potassium phosphate buffer (pH 6.7), purified by reversed-phase HPLC as described above, and then analyzed by electron impact mass spectrometry (Fisons VG Tribrid; EI+ mode; 70 eV). LAS and LAS desulfonation products were separated by reversed-phase HPLC on a Spherisorb ODS2 column (250 by 4.6 mm; particle size, 5 μm); the mobile phase was 0.11 M $NaClO_4$ with an acetonitrile gradient (0 to 100%, vol/vol), and compounds were detected spectrophotometrically at 225 nm (20, 27). Proton nuclear magnetic resonance (^{1}H-NMR) spectra were determined with a Bruker 400-MHz instrument. Chemical shifts (in parts per million) were determined relative to a tetramethylsilane internal standard.

Sulfate levels were measured by ion chromatography, using an anion-exchange column (an AS9 column with an AG9 guard column; Dionex, Sunnyvale, Calif.); the compounds were eluted isocratically by using 1.7 mM $NaHCO_3$–1.8 mM Na_2CO_3 and were detected conductimetrically. Cells were lysed to release sorbed sulfonates by adjusting the NaOH concentration in the total culture to 0.3 M, shaking the preparation at 30°C for 18 h, and then neutralizing the preparation with HCl and centrifuging it at 10 000 × *g* for 10 min. Total cell protein levels were measured by a modification of the Lowry method (17), using bovine serum albumin as the standard. All glassware used in sulfate-limited experiments was washed with 3 M HCl and rinsed thoroughly with distilled water before use.

RESULTS

Utilization of arylsulfonates by *P. putida* S-313. *P. putida* S-313 was supplied with a range of arylsulfonates as sole sources of sulfur (50 μM) in succinate minimal medium. Studies of the growth yields of *P. putida* S-313 revealed that sulfur was the growth-limiting element in this growth medium up to a concentration of 80 μM (2a). All arylsulfonates were stable in sterile growth medium. Growth was observed with all of the LAS-related monosulfonic acids tested (Table 1) except LAS itself. Strain S-313 was also able to utilize a monosulfonate azo dye, Orange II (C.I. 15510), whereas a related disulfonated dyestuff, Acid Red I (C.I. 18050), and the disulfonated stilbene optical brightening agent 4,4′-bis(2-sulfostyryl)biphe-

TABLE 3. Products from the utilization of arylsulfonates by *P. putida* S-313[a]

Sulfur source for growth	Product deduced from mass spectrometry and NMR data	Analytical data
p-Toluenesulfonate	*p*-Cresol	*m/z*: 108 (M^+), 107 (M^+-H), 80 (M^+-CO)
Sulfophenylbutyrate	2-(4-Hydroxyphenyl)butyrate	*m/z*: 180 (M^+), 135 (M^+-COOH), 107(M^+-COOH, C_2H_5)
Sulfophenyloctane	4-Octylphenol	*m/z*: 206 (M^+), 107 (M^+-C_7H_{15})
DATS	4-Butyl-1-methyl-6-hydroxytetralin	*m/z*: 218 (M^+), 203 (M^+-CH_3), 175 (M^+-CH_3, CO), 161 (M^+-C_4H_9), 147 (M^+-C_3H_7, CO), 133 (M^+-C_4H_9, CO)
Orange II	4-[(2-Hydroxy-1-naphthalenyl)azo]phenol	*m/z*: 264 (M^+), 171, 93 (fragmentations adjacent to diazo bond), 143, 65 (*m/z* 171-CO, 93 - CO) ^{1}H-NMR: δ (d_6-DMSO) 6.89 (d, 8.3 Hz, 3-H), 7.16 (d, 9.0 Hz, 3′-H), 7.62 (t, 6.9 Hz, 7′-H), 7.64 (t, 7.6 Hz, 6′-H), 7.87 (d, 8.8 Hz, 2-H), 7.88 (d, 7.4 Hz, 5′-H), 7.94 (d, 9.0 Hz, 3′-H), 8.73 (d, 8.3 Hz, 8′-H)[b]

[a] *P. putida* S-313 was grown in succinate minimum medium containing different sulfur sources. Four products were identified by gas chromatography-mass spectrometry; the product obtained from the dyestuff Orange II was tentatively identified by mass spectrometry, and the identity was confirmed by ^{1}H-NMR. The major differences between the NMR spectra of the educt and the product were observed for the proton originally adjacent to the sulfonate group in Orange II (2-H: shift from δ = 7.80 to 6.89 ppm) and the proton adjacent to the hydroxy group on the naphthyl ring (3′-H: shift from δ = 6.91 to 7.16 ppm).
[b] DMSO, dimethyl sulfoxide.

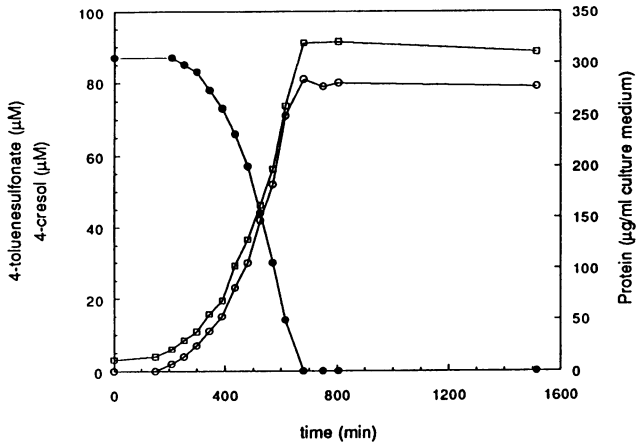


FIG. 2. Utilization of 4-toluenesulfonate as a sole sulfur source by *P. putida* S-313. The cells were grown aerobically at 30°C in succinate-salts medium. Samples were withdrawn periodically and assayed for total protein content (□) by using a modified Lowry assay and for 4-toluenesulfonate (●) and 4-cresol (○) contents by HPLC.

nyl were not degraded (Table 1). The nonutilization of aromatic disulfonates was not due to a general inability of *P. putida* S-313 to take up such compounds, because this organism has been shown to utilize aromatic aminodisulfonates (47) and the simple disulfonate Tiron as sulfur sources (Table 1).

The growth yields observed with most arylsulfonates when they were used as sulfur sources were approximately 2.8 kg of protein per mol of S, the value observed with sulfate (Table 1), which was completely removed from the growth medium. We assume that the arylsulfonate sulfur in toluenesulfonate, sulfophenylbutyrate, sulfophenyloctane, and Orange II was assimilated essentially completely into cell material.

Identification and quantification of products obtained from the desulfonation of arylsulfonates by *P. putida* S-313. Products in outgrown cultures were extracted into ether and analyzed by gas chromatography-mass spectrometry. One product was detected for each of the first four utilizable substrates listed in Table 1, and in each case the product was identified as the corresponding phenol (Table 3). The product obtained from Orange II could not be identified under these conditions, presumably because of decomposition of the azo bond at the high temperature of the gas chromatography injector. A product was detected by HPLC, however, and this product exhibited a slight shift in absorption spectrum compared with the starting material (λ_{max} for Orange II, 485 nm; λ_{max} for the product, 423 and 456 nm). This product was purified by reversed-phase HPLC and was analyzed by direct probe insertion mass spectroscopy. The mass spectrum obtained was consistent with the mass spectrum of the corresponding phenol (Table 3); this finding was confirmed by ^{1}H-NMR (Table 3).

Direct quantification of the stoichiometry of desulfonation could be done only for the utilization of *p*-toluenesulfonate, because the product of this reaction, *p*-cresol, was the only compound commercially available. *p*-Toluenesulfonate was totally consumed during growth (Fig. 2), as anticipated from the growth yield (Table 1), and >90% of the aromatic ring was recovered as *p*-cresol (Fig. 2). We assume that this represented complete recovery of the organic product as the phenol. We expected complete substrate utilization and release of the organic moiety as the phenol in all cases where simple mass balances are indicated in Table 1, but there were two possible exceptions (Table 1). We suspect that the disulfonate Tiron is subject to only one desulfonation (the measured yield was 2.9 kg of protein per mol of S, whereas about 5.6 kg was expected). In the case of DATS, we were not sure of the purity of the traces of material which we had, and thus it was not clear whether incomplete utilization, utilization of only one isomer, or total utilization of the available material took place.

Binding of LAS to *P. putida* S-313. Negligible growth was observed when strain S-313 was supplied with LAS as a sole sulfur source (Table 1). Nevertheless, the substrate largely disappeared from the culture solution during incubation. No adsorption of LAS to the glass growth vessels was observed (14), and we suspected that the long-chain arylsulfonates were either taken up by the cells or adsorbed to their surfaces. Cultures were grown with a mixture of excess sulfate (200 μM) (the preferred sulfur source [2a]) and LAS and then treated with 0.3 M NaOH to lyse the cells. An analysis of the neutralized hydrolysate by HPLC revealed that each LAS component in the mixture was completely recovered. This demonstrated that substrate disappearance in the absence of growth was indeed due to sorption onto or into the cells, although the exact mechanism was not determined. Control experiments showed that the LAS mixture was stable to the NaOH treatment. This binding was presumably the same phenomenon observed by Giger et al. (13) in sewage treatment plants. A similar level of substrate disappearance (84%) in the absence of growth was observed with 4,4′-bis(2-sulfostyryl)biphenyl but was not investigated further (cf. 34).

Enrichment of a culture to desulfonate LAS. Sulfate-free minimal medium containing commercial LAS as a sole source of sulfur was inoculated with washed material from a municipal activated sludge plant. A stable, LAS-utilizing mixed culture, culture L6, was obtained after three subcultures into homologous medium. This culture grew with succinate, glycerol, or glucose as a sole source of carbon. We chose to work with 10 mM glucose and 50 μM LAS; under these conditions the mixed culture attained its highest level of turbidity, and the stationary phase was reached within 2 days. A microscopic examination of culture L6 revealed the presence of one dominant organism, a fluorescent, motile, rod-shaped bacterium, which we were not able to isolate as a pure culture.

Specificity of LAS degradation by culture L6. The molar growth yield observed after growth of culture L6 in the presence of 50 μM LAS was significantly less than the yield obtained with sulfate (data not shown). An analysis of the supernatant fluid from outgrown cultures revealed that the LAS completely disappeared, but alkaline hydrolysis of the whole culture revealed that sorption was responsible for part of this disappearance (Fig. 1B and C), as observed with *P. putida* S-313 (see above).

Degradation was isomer specific (Table 2). The fastest degradation was observed with the 5-(4-sulfophenyl)-substituted isomers tested (m = 3) (Table 1), and there was progressively less desulfonation the closer the sulfonophenyl group was to the end of the alkyl chain. The total length of the chain also had a clear effect; shorter chains (C_{11}) were utilized better than longer chains (C_{13}) (Table 2).

Products from LAS were obtained by subjecting neutralized hydrolysates to ether extraction. This material was examined by HPLC, and a set of peaks with longer retention times than the LAS peak was detected (Fig. 1D). The pattern of peaks in this product mixture corresponded approximately to the amounts of the LAS isomers utilized (Table 2), assuming that all products had similar molar absorbance coefficients. The yield of each isomer was calculated relative to its abundance in

TABLE 4. Representative products obtained from LAS desulfonation by mixed culture L6[a]

Product	Analytical data
5-(4-Hydroxyphenyl)$C_{11}H_{23}$	*m/z*: 248 (M^+), 191 (M^+-C_4H_9), 163 (M^+-C_6H_{13}), 107 (M^+-C_6H_{13}, C_4H_9)
4-(4-Hydroxyphenyl)$C_{11}H_{23}$	*m/z*: 248 (M^+), 205 (M^+-C_3H_7), 149 (M^+-C_7H_{15}), 107 (M^+-C_7H_{15}, C_3H_7)
3-(4-Hydroxyphenyl)$C_{11}H_{23}$	*m/z*: 248 (M^+), 219 (M^+-C_2H_5), 135 (M^+-C_8H_{17}), 107 (M^+-C_8H_{17}, C_2H_5)
2-(4-Hydroxyphenyl)$C_{11}H_{23}$	*m/z*: 248 (M^+), 107 (M^+-C_9H_{19}, CH_3)
5-(4-Hydroxyphenyl)$C_{13}H_{27}$	*m/z*: 276 (M^+), 219 (M^+-C_4H_9), 163 (M^+-C_9H_{19}), 107 (M^+-C_9H_{19}, C_4H_9)
4-(4-Hydroxyphenyl)$C_{13}H_{27}$	*m/z*: 276 (M^+), 233 (M^+-C_3H_7), 149 (M^+-$C_{10}H_{21}$), 107 (M^+-$C_{10}H_{21}$, C_3H_7)

[a] Mixed culture L6 was grown by using commercial LAS as the sole source of sulfur. The degradation products were partially purified by reversed-phase HPLC and were identified by gas chromatography-mass spectrometry.

the starting mixture (Table 2). The HPLC eluate which contained the products was collected, concentrated, and examined by gas chromatography-mass spectrometry. The main components were identified as long-chain alkylphenols corresponding to components of the LAS educt (Table 4).

Effect of sulfate on desulfonation of LAS. Mixed culture L6 was grown by using a mixture containing excess sulfate (200 μM) and LAS (50 μM) as sulfur sources for growth. HPLC analysis revealed that every isomer of the LAS was recovered quantitatively after alkaline lysis of the cells. The presence of sulfate therefore either suppresses the synthesis or inhibits the activity of a desulfonative enzyme(s) in the cell, as previously observed for *P. putida* S-313 (18, 47).

DISCUSSION

We obtained conclusive evidence that direct bacterial desulfonation of components of LAS surfactants to the corresponding phenols occurs (Tables 2 and 4 and Fig. 1), and these phenols are not further metabolized under the conditions which we used (Fig. 2). By analogy to desulfonation in *P. putida* S-313, we assume that the reaction involves a monooxygenase (see below). This is the first evidence for direct desulfonation of commercial LAS (31, 36, 40), although it has been claimed that the reaction occurs when (4-sulfophenyl)undecane (43) is used as a carbon source for growth.

The growth yields in Table 1 represent mass balance values for the sulfonate moiety, which was recovered effectively as methionine and cysteine in cell protein. Our data extend the data reported previously for naphthalenesulfonates and benzenesulfonates (47). As before, the products were the phenols resulting from simple desulfonation of the starting sulfonates; no modification of the carbon skeletons of the molecules was observed (Table 3). It is thought that degradation of aromatic sulfonates by *P. putida* S-313 under sulfur-limited growth conditions is catalyzed by a broad-substrate-range monooxygenase system (47) which has never been observed in vitro (2a).

The apparently simple desulfonation process described above is not, however, part of the metabolism of LAS that is thought to occur in an activated sludge plant. The generally accepted pathway of LAS degradation assumes that the material provides a carbon and energy source for growth of a consortium of bacteria. Initial ω-oxygenation of the alkyl chain is followed by a series of β-oxidation steps which result in a sulfophenylcarboxylate intermediate. Desulfonation and ring cleavage of the aromatic moiety then follow in unknown order in another organism (31, 36, 40). Biodegradation of LAS by this pathway is faster for longer-chain isomers (40), and there is a clear preference for positional isomers that have the sulfophenyl ring near the end of the alkyl chain. This preference has been termed the "distance principle"; the greater the distance between the xenobiotic arylsulfonate moiety and the site of initial ω-oxygenation, the more rapid the biodegradation (40). For culture L6 utilizing LAS as the sulfur source(s), however, the reverse was observed; the shorter-chain isomers were utilized better than the longer-chain isomers, and the 2-substituted LAS were desulfonated considerably less rapidly than the centrally substituted isomers (Table 2).

The reaction which we observed has the potential advantage that the most xenobiotic group in LAS, the sulfonate moiety, is removed, thus presumably making the product available to less specialized microorganisms that are able to degrade aromatic rings. The reaction specificity also tends to complement the specificity observed for LAS biodegradation in the past and may have potential for removing the less-well-biodegraded components of commercial LAS in practical applications. However, the usefulness of this reaction for wastewater treatment is complicated considerably by the fact that it stops completely in the presence of sulfate (Fig. 1) (47). It has been observed that the synthesis of a number of proteins is regulated by the presence of sulfate or cysteine in *P. putida* and other species, as part of the sulfate starvation-induced stimulon (18). If the enzyme system(s) responsible for LAS desulfonation in culture L6 is also controlled in this way, then further investigation and manipulation of the regulatory systems concerned may be required before this novel mechanism of LAS degradation can be employed practically.

The simple removal of the xenobiotic sulfonate group described above is especially important in the case of DATS, since these compounds are poorly degraded under carbon-limited conditions (12). Facile microbial degradation of the dyestuff Orange II is also unusual and appears to be specific to the sulfate-limited conditions provided. This compound is largely recalcitrant to aerobic, carbon-limited degradation because of the xenobiotic nature of the sulfono substituents; the corresponding carboxy dyestuff is readily broken down as a carbon source (21). Several groups of workers have observed reductive cleavage of the azo bond in Orange II under anaerobic conditions (8, 28, 44, 45), and recently Haug et al. (15) described complete mineralization of a related dyestuff by using an anaerobic-aerobic treatment cycle. Under sulfate-limited conditions, the initial aerobic removal of the sulfonate group, as described in this paper, should yield a product which is much more amenable to further aerobic degradation than is the sulfonated educt (21).

Sorption of LAS to biomass or to inorganic material has been observed frequently in the past with sewage sludge (26, 35) and with bacterial cultures (see references 40 for a review). LAS sorption increases with increasing chain length both for bacteria (*Escherichia coli*, *Staphylococcus aureus* [40]) and for the biological agent-inorganic compound mixture which makes up sludge (14), although binding is generally better for the latter (40). These findings were confirmed by the sorption data obtained for culture L6 (Table 2). Substitution position had a negligible effect on sorption of LAS by culture L6. Whereas in

previous studies workers had to use nonbiodegrading organisms (e.g., *E. coli*) in order to distinguish sorption processes from biodegradation (40), in the system described in this paper the addition of sulfate to the growth medium was sufficient to prevent biodegration from taking place, allowing study of sorption processes separately from LAS metabolism processes.

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